Short report

Gonorrhoea: auxotypes, serovars, and clinical manifestations among female sex workers from Kinshasa, Zaïre

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The main question in this paper was to look at the distribution of auxotypes and serovars of *Neisseria gonorrhoeae* and check whether they correlate with clinical symptoms/signs among female sex workers (FSW) from Kinshasa, Zaïre. The subjects were 1233 FSW enrolled in a cross sectional study on STDs and HIV infection in 1988; 771 of them were followed prospectively for a median duration of 23 months. At each visit, clinical symptoms and signs of cervicitis were recorded and the subjects were screened for gonococcal and chlamydial infection. The predominant auxotypes were prototrophic (35·2%), proline requiring (29·6%), and proline requiring phenylalanine inhibition (19%). Serovars 1A-6 (42·5%) and 1B-1 (16·7%) were the commonest. Infection with auxotype prototrophic and phenylalanine inhibition (Proto/Phenal^{i}) was significantly associated with both mucopurulent cervicitis and pelvic inflammatory disease; (OR = 8·9; p = 0·002 and OR = 19·9; p = 0·002; respectively). Despite the few associations found in this study, there was no clear pattern linking clinical manifestations to auxotype/serovar profiles.
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Keywords: *Neisseria gonorrhoeae*; auxotypes; serovars; symptoms/signs

Introduction

It is well known that the epidemiology of *Neisseria gonorrhoeae* infection is influenced by auxotypes and serovars that are structurally and antigenically different.[1 2] Geographical, temporal, and ethnical variations of auxotypes and serovars have been documented.[3 4] In industrialised countries, the availability of serotyping has allowed detailed epidemiological and clinical studies of gonococcal infection over the past 20 years. However, only a few studies have been conducted in developing countries of tropical Africa on gonococcal resistance according to the serological classification of isolates.[5] To our knowledge, no survey has been published from this part of the world on the expression of gonococcal disease according to the phenotype of isolates. This study was undertaken to look at the distribution of auxotypes and serovars of *N gonorrhoeae* and to check whether they correlate with clinical symptoms/signs among female sex workers from a developing country, Zaïre.

Methods

In 1988, 1233 female sex workers were enrolled in a cross sectional study, screened for STDs and HIV antibodies in Matonge, Kinshasa by the Projet/SIDA Zaïre. This population has been described extensively elsewhere.[6] Briefly, 771 of these women were followed prospectively, once every month for a median duration of 23 months (range 3–36 months). Demographic characteristics, medical and sexual history were recorded by a nurse in a face to face interview. The nurse also recorded information about urogenital symptoms (vaginal discharge, lower abdominal pain, and vulvar itching). A physician performed a gynaecological examination to record clinical signs (vaginal discharge, cervical motion tenderness, cervix erosion, and mucopurulent cervicitis).

Samples from gynaecological examination were tested for *N gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and yeasts. Gonococci were isolated on modified Thayer-Martin medium incubated in a candle extinction jar at 35°C for 24–48 hours. Isolates were identified on the basis of typical colony morphology, oxidase reaction, and sugar utilisation patterns. Strains were tested for their requirements for proline, arginine, hypoxanthine, uracil, methionine, amino acids, vitamins, and for inhibition by phenylalanine according to the auxotyping method described by Hendry and Stewart.[2] For serotyping, strains were tested by coagglutination with monoclonal antibodies. The Knapp's serovar nomenclature was used.[1]

Data analysis concerned 1094 infective episodes with *N gonorrhoeae*; 286 of them were diagnosed at enrolment whereas 808 were incident episodes (defined as a patient presenting a positive test at a follow up visit with a negative test at the previous visit or with a suitable treatment in the event of a positive test at the previous visit). Statistical analysis to compare the prevalence of recorded symptoms/signs measured according to different auxotypes, serovars, and auxotype/serovar classes was performed using χ^2. When the proportions were significantly different, we calculated the

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Table 1 Auxotypes, serovars of Neisseria gonorrhoeae among female sex workers from Kinshasa, Zaïre

Auxotype (n = 622)	*No of strains (%)*	*Serovar (n = 659)*	*No of strains (%)*
Proto	219 (35·2)	Serogroup IA	348 (52·8)
Proto/Phenal^i	11 (1·8)	IA0	2 (0·3)
Pro$^-$	184 (29·6)	IA1	12 (1·8)
Pro$^-$/AA$^-$/Phenal^i	1 (0·2)	IA2	12 (1·8)
Pro$^-$/Phenal^i	118 (19·0)	IA3	2 (0·3)
Pro$^-$/Arg$^-$	76 (12·2)	IA4	21 (3·2)
Pro$^-$/Hypx$^-$/Ura$^-$	1 (0·2)	IA5	8 (1·2)
Pro$^-$/Vit$^-$	2 (0·3)	IA6	280 (42·5)
Arg$^-$	3 (0·5)	IA8	9 (1·40)
Pro$^-$/Arg$^-$/AA$^-$	1 (0·2)	IA17	2 (0·3)
Met$^-$	1 (0·2)	Serogroup IB	273 (41·4)
AA$^-$	1 (0·2)	IB0	7 (1·0)
		IB1	110 (16·7)
		IB2	5 (0·8)
		IB3	34 (5·2)
		IB4	7 (1·0)
		IB5	17 (2·6)
		IB6	15 (2·3)
		IB7	67 (10·2)
		IB8	2 (0·3)
		IB17	1 (0·2)
		IB23	1 (0·2)
		IB24	7 (1·0)
		Not typable	38 (5·8)

Proto = prototrophic; Phenal^i = phenylalanine inhibition; Pro$^-$ = proline requiring, AA$^-$ = amino acids requiring; Arg$^-$ = arginine requiring; Hypx$^-$ = hypoxanthine requiring; Ura$^-$ = uracil requiring; Vit$^-$ = vitamins requiring; Met$^-$ = methionine requiring.

odds ratios and p values using a 2 × 2 table comparing each auxotype/serovar with all other considered together (Fisher's bilateral exact test). When significant associations were found in crude data, we performed analysis restricted to women not infected by *C trachomatis*. Finally, we calculated the prevalence of auxotype/serovar classes every 6 months during the study period, and we limited comparison (using χ^2) to the first two 6 month periods, since the majority of infections occurred in the first year.

Results

Clinical symptoms/signs of cervicitis were recorded respectively in 542 (87·1%) of the 622 episodes with gonococcal auxotype data, and in 577 (87·6%) of the 659 episodes with gonococcal serovar data.

As shown in table 1, the predominant auxotypes identified were prototrophic (Proto), proline requiring (Pro$^-$), proline requiring/phenylalanine inhibition (Pro$^-$/Phenal^i) and proline/arginine requiring (Pro$^-$/Arg$^-$). These four commonest strains accounted for 96% of infections. The three most frequent serovars which encompassed 69·4% of infections were 1A-6, 1B-1, and 1B-7. Of the 659 strains serotyped, 348 (52·8%) were serogroup 1A, 273 (41·4%) belonged to serogroup 1B, and 38 (5·8%) strains were ungroupable.

Combining the two classification systems, a total of 63 distinct auxotype/serovar classes were identified. The prevalence of the majority of these classes remained stable over the study period with the exception of Proto/1B-7 and Pro$^-$/1A-6 classes that had changed dramatically in the second 6 months. The former was increased by a factor of 5 (from 4·6% to 23·6%; $p < 0·01$) and the second decreased very significantly (from 26·1% to 10·6%; $p < 0·01$).

Table 2 summarises the prevalence of recorded clinical symptoms/signs according to different auxotypes. Mucopurulent cervicitis (MPC) was more common in women infected with auxotype Proto/Phenal^i than in women with all other strains; (5/10 (50%) women infected with auxotype Proto/Phenal^i had MPC *v* 54/532 (10·2%) women infected with all other auxotypes (OR = 8·9; p = 0·002)). Women infected with the same Proto/Phenal^i auxotype had more pelvic inflammatory disease (PID) than those infected with all other strains (50% of the women infected with this auxotype had PID *v* 4·8% for all other auxotypes (OR = 19·9; p = 0·002)). When we restricted analysis to women without chlamydial infection, MPC and PID remained significantly associated with auxotype Proto/Phenal^i.

None of the symptoms/signs was significantly associated with any particular serovar. However, serovar 1B-7 was significantly associated with the absence of signs (44/64 (68·8%) women infected with serovar 1B-7 did not present any sign *v* 229/513 (44·6%) for all other serovars (OR = 2·2; p = 0·003)).

Discussion

Our data show that the most frequent auxotypes were prototrophic and proline requiring. We noticed that the predominance of these two auxotypes was also reported elsewhere in Africa (Gabon) and in several studies performed in America and Europe.[5-8] However, auxotypes Arg$^-$ Hyp$^-$ Ura$^-$ and Pro$^-$ Cit$^-$ Ura$^-$ Hypx$^-$ which have been isolated in Europe and America were not isolated in our study population. We also found auxotype Pro$^-$Phenal^i very frequent (19%). This auxotype was not isolated in other studies of which

Table 2 Prevalence of clinical symptoms and signs of Neisseria gonorrhoeae infection according to auxotype among female sex workers from Kinshasa, Zaïre

	% of women with symptom or sign by auxotype						
	Proto (n = 191)	*Proto/Phenal^i (n = 10)*	*Pro$^-$ (n = 161)*	*Pro$^-$/Phenal^i (n = 105)*	*Pro$^-$/Arg$^-$ (n = 63)*	*Others (n = 12)*	*p* Value*
Symptoms:							
Vaginal discharge	17·3	10·0	15·5	5·7	15·9	16·7	0·14
Lower abdominal pain	27·8	50·0	27·3	35·2	39·7	25·0	0·22
Vulvar itching	18·3	10·0	22·4	15·2	17·5	25·0	0·68
Signs:							
Vaginal discharge	42·4	70·0	44·7	41·9	58·7	50·0	0·14
Cervix erosion	13·0	0·0	11·8	6·7	9·5	8·3	0·48
Mucopurulent cervicitis	9·9	50·0	11·2	3·8	19·0	8·3	< 0·01
Cervical motion tenderness	7·9	10·0	6·8	11·4	7·9	8·3	0·86

Proto = prototrophic; Phenal^i = phenylalanine inhibition; Pro$^-$ = proline requiring; Arg$^-$ = arginine requiring; Others = different infrequent auxotypes. p* Value from χ^2 test on the 2 × 6 table comparing the proportions of women with each specific symptom/sign by auxotype.

we are aware since strains were not tested for the inhibitory activity of phenylalanine.

In industrialised countries, the serovars of serogroup B seem to predominate whereas in our study, as in Gabon, more than half of the strains were serogroup A. Indeed, in Spain, serovars of serogroup B accounted for 93·5% of all strains.[8] This serogroup also predominated in Canada (66·8%) and in Sweden (70%).[2 9]

We observed dramatic changes in the gonococcal population infecting the cohort over the study period with the introduction of new strains and loss of other strains. This has been previously reported.[3] The significant loss of certain strains supports the notion of gonococcal strain specific acquired (partial) immunity, as proposed by Plummer and colleagues who reported that women experiencing an infection with a specific gonococcal serovar were at 2- to 10-fold reduced risk of reinfection with the same strain.[10]

Despite the fact we made multiple comparisons, we found that only a few auxotypes/serovars of *N gonorrhoeae* were significantly associated with clinical manifestations of gonococcal infection and the strength of some associations may suggest that they did not occur by chance only. Indeed, we found that auxotype Proto/Phenal^{i} was strongly associated with both MPC and PID. However, this apparently increased virulence of auxotype Proto/Phenal^{i} should be interpreted with caution because of the small number of women infected with this strain (only 11 of the 622 auxotypes) and the impossibility of comparing our results with those reported by other authors since this auxotype was not isolated in their studies.

In summary, we did not identify many associations between auxotypes/serovars of *N gonorrhoeae* and symptoms/signs. To compare the associations we observed and to obtain a better understanding of the epidemiology of gonococcal infection in developing countries, there is a need for additional studies using similar methods distinguishing between strains of *N gonorrhoeae* (including testing for phenylalanine inhibition) in areas where the same isolates can be found.

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